FEBS Letters 586 (2012) 2925–2931

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

The p38 and Hog1 SAPKs control cell cycle progression in response to environmental stresses

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ARTICLE INFO

Article history:
Received 4 July 2012
Revised 11 July 2012
Accepted 12 July 2012
Available online 20 July 2012

Edited by Miguel De la Rosa, Felix Wieland and Wilhelm Just

Keywords:
Stress-response
SAPK
Cell cycle regulation

ABSTRACT

In response to environmental stresses, cells need to activate an adaptive program to maximize cell progression and survival. Stress-activated protein kinases (SAPK) are key signal transduction kinases required to respond to stress. Prototypical members of SAPKs are the yeast Hog1 and mammalian p38. Upon stress, those enzymes play a critical role in mounting the adaptive responses to stress such as the regulation of metabolism and the control of gene expression. In addition, a major function of SAPKs in response to stress is to modulate cell cycle progression. In this review, we focus on the role of Hog1 and p38 in the control of cell cycle progression in response to environmental stresses.

1. Introduction

Cyclin-dependent kinase (Cdk) complexes drive cell cycle progression. In the budding yeast *Saccharomyces cerevisiae*, a sole Cdk, Cdc28 (which is the functional homologue of Cdk1) controls cell cycle by association with specific cyclins which confer substrate specificity [1–4]. In higher eukaryotes, multiple Cdk associate with multiple cyclins to regulate cell cycle progression [5]. A second layer of cell cycle control is orchestrated by proteins involved in the fine-tune regulation of these cyclin-Cdk complexes, including a vast number of cell cycle regulators which control cyclin transcription, translation, localization and degradation as well as protein cyclin-Cdk inhibitors [6,7]. All those factors ensure the proper coordination of hundreds of molecular events during cell cycle progression.

On top of that, an extra layer of control ensures the correct completion of every phase of the cell cycle before entering into the next one. This function is carried out by checkpoint proteins which are involved in controlling processes such as morphogenesis, cell size, DNA replication or spindle-assembly [8]. In general, checkpoint responses consist in a transient cell cycle arrest to provide time to the cell to overcome primary problems (e.g. an incomplete or aberrant cell cycle event). Mutations in checkpoint proteins might result, for instance, in misscoordination between the mitotic and the morphogenetic cell cycle, aneouploidy or aberrant DNA structures. In yeast and in other unicellular organisms all these defects may lead to cell death, whereas in metazoans, they are causally related with early and late stages of cell transformation and tumorigenesis [9,10]. Therefore, checkpoint pathways can be defined as surveillance mechanisms that ensure the proper coordination and completion of cell cycle events, essential to preserve cell integrity and genomic stability.

Although being part of the cell cycle regulation core, checkpoint signaling pathways have a particularly important role in response to internal or external toxic agents. To date, the most well studied kind of stress that activates a checkpoint pathway is the genotoxic stress caused by cell metabolism (reactive species of oxygen, ROS), exposition to ultraviolet light (UV) that lead to DNA damage accumulation [11,12] as well as replicative stress, which takes place during S-phase when the replication fork cannot progress because of the absence of the DNA precursors, dNTP, or the missfunction of the DNA polymerases. In response to DNA damage or replication stress, cells activate the DNA damage checkpoint pathway to arrest cell cycle, providing time to repair the DNA damage or to overcome the replication stress [8,13]. However, cells must cope with other stresses in addition to that of genotoxic stress that poses a risk for cell survival. For instance, cells are exposed to changing environmental conditions, such as changes in pH, nutrient availability, temperature, and osmolarity that directly affect cell homeostasis and physiology.

Cells have evolved a number of signal transduction pathways that serve to adapt and survive to stress. Yeast and mammals have

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0014-5793
http://dx.doi.org/10.1016/j.febslet.2012.07.034

a conserved family of mitogen-activated protein kinase (MAPKs) that sense and respond to extracellular environmental changes known as stress-activated signaling pathways (SAPKs). Activation of SAPKs leads to generation of a set of adaptive responses that involves the modulation of several physiological processes such as changes in gene transcription, cell metabolism, protein translation and cell cycle progression [14–16].

It has been known for a long time that environmental stresses lead to a transient cell cycle arrest and that the bypass of this cell cycle delay is detrimental for cell survival [17–22]. Thus, cells activate checkpoint surveillance mechanisms in response to extracellular stimuli to modulate cell cycle progression and to permit adaptation to changing environmental conditions. In most of the cases, the proteins and the molecular mechanisms involved in those checkpoint responses to environmental cues remain to be elucidated. Here, we review the latest studies on how osmostress impacts on cell cycle progression and discuss the importance of novel checkpoint mechanisms in preserving genomic integrity and cell viability from budding yeast to mammals.

2. The HOG/p38 stress signaling pathways

Exposure of cells to osmostress results in rapid and transient activation of SAPKs. In budding yeast, the HOG (high osmolarity glycerol) pathway is the main mediator of cellular adaptation upon osmostress and it is one of the best characterized SAPK cascades in eukaryotes (revised in [23–26]) (see Fig. 1). Two independent sensor branches triggers the activation of the HOG pathway: the Sln1 branch and the Sho1 branch. Each sensor branch is sufficient to trigger the activation of the pathway, although the Sln1 branch is more prominent in pathway control and display higher sensitivity to respond faster and over a wide range of osmolarity changes [27–29]. The core of the pathway comprises a layer of three MAPKKK (Ssk2, Ssk22 and Ste11) which activate the unique Pbs2 MAPKK, which in turn phosphorylates and activates the Hog1 MAPK [30]. In mammalian cells, both the architecture and the main players of the pathway are highly conserved, being p38 a Hog1 homolog [31,32] (see Fig. 1). It is worth mentioning that while Hog1 is mainly activated upon osmostress, and it would play only a minor role in response to other stresses for instance heat, oxidative and unfolded protein response (UPR) stresses [33–36], p38 is activated by multitude of external stimuli such as cytokines, DNA damage, oxidative and heat stresses, osmostress, etc. The central core of the pathway is similar to HOG albeit the molecular activation mechanisms that lead to its activation to stress are not well defined. Moreover, in contrast to Hog1, p38 function is crucial not only for the acute response to cellular insults but it also plays key roles in controlling differentiation, proliferation, apoptosis, cell morphology and immune response [16,37].

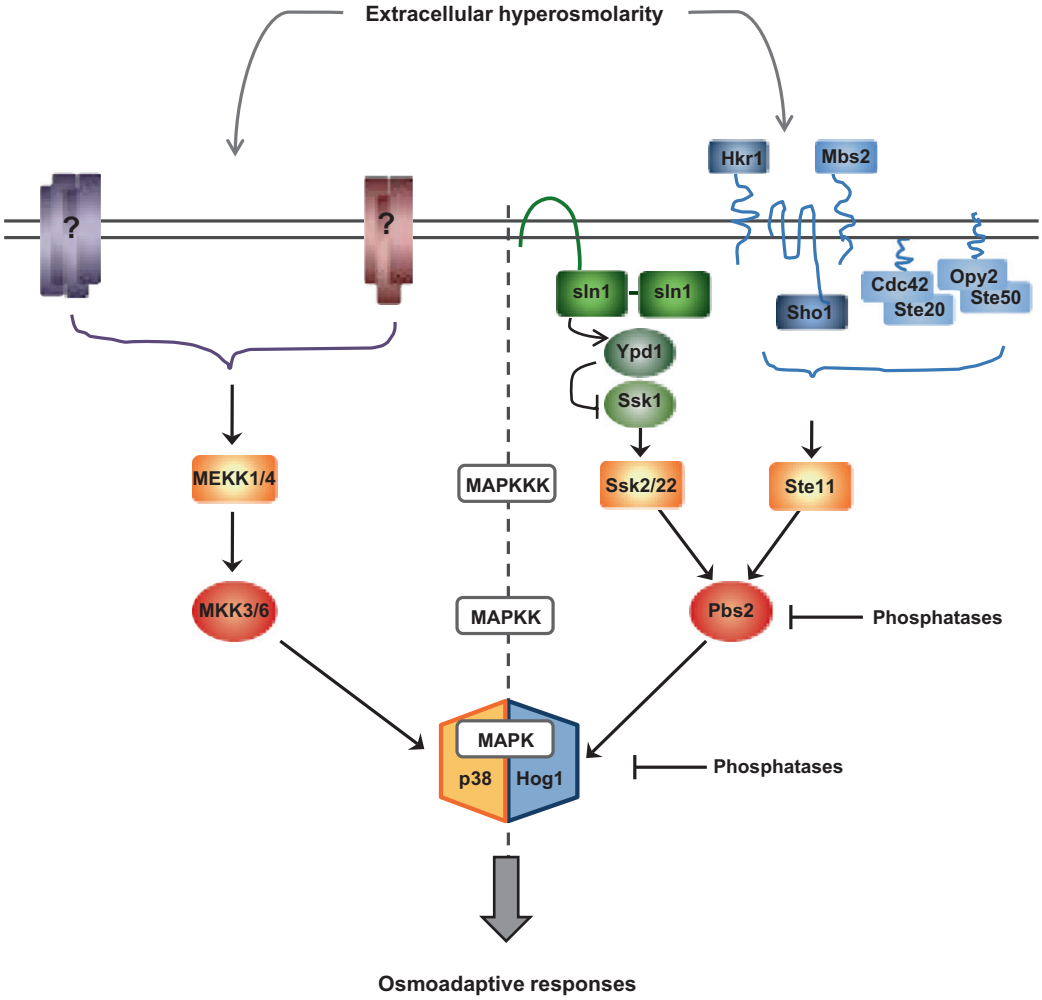

Fig. 1. Schematic diagram of the Hog1/p38 SAPK pathways. In response to osmostress different osmosensors mechanisms become activated when cells detect changes in osmolarity. While in mammalian cells (left panel) the osmosensor complexes have not been clearly defined, in budding yeast (right panel), two independent osmosensing mechanisms, the Sln1 and Sho1 branches, are activated upon osmostress. Activation of those osmosensors complexes leads to the activation of the MAPKKKs, MEKK1/4 in mammalian cells and Ssk2/22 and Ste11 in budding yeast, which in turn activate the MAPKKs Mkk3/6 and Pbs2 respectively. Activated MKK4/6 and Pbs2 phosphorylates and activates the p38 and Hog1 MAPKs respectively, which trigger the osmoadaptive response by phophorylation of multiple substrates.

In response to osmostress, Hog1/p38 activation elicit the program for cell adaptation required for cell survival including changes in gene transcription, cell metabolism, protein translation and cell cycle progression [23–26]. In recent years, Hog1 and p38 have been involved in modulation of cell cycle progression by controlling different phases of the cell cycle such as G1/S, S and G2/M. In the following sections, we describe how Hog1 and p38 SAPKs modulate the timing of cell cycle progression in response to stress by different molecular mechanisms and their relevance on cell survival upon stress.

3. Regulation of G1 by SAPKs upon stress

In budding yeast, progression through G1 is driven by the G1 cyclins (Cln1, Cln2 and Cln3) and the S-phase entry is triggered by the S phase cyclins Clb5 and Clb6 (reviewed in [38]). Activation of Hog1 by exposure of cell to high osmolarity (or due to activation of the upstream components of the MAPK pathway) results in a transient arrest in G1. This cell cycle delay in G1 involves the stabilization of the CDK inhibitor Sic1 [39–41] (see Fig. 2). Sic1 is the main Clb-CDK inhibitor and it is essential to fine tune the precise time of S-phase entry. Sic1 keeps the Clb5,6-Cdc28 inhibited and sets a threshold for the Clb-Cdc28 activation during the G1/S transition [38]. Sic1 expression is triggered when cells exit mitosis and peaks at early G1. When cells progress through G1, Cln-Cdc28 activity increases up to late G1, and it phosphorylates the N-terminal domain of Sic1, which targets it for ubiquitination by Cdc4 and degradation by the proteasome [42]. Once Sic1 is degraded, Clb5,6-Cdc28 is released from the inhibition and triggers DNA replication [43]. Upon osmostress, Hog1 physically interacts with and phosphorylates Sic1 in one single residue (Thr173) at the carboxyl terminus of Sic1. This phosphorylation interferes with the association of Sic1 with Cdc4, reduces its degradation and leads to cell arrest in G1. The stabilization of Sic1 and the consequent Hog1-dependent G1 arrest is essential for the adaptive response to osmostress, since cells lacking Sic1 or carrying a non-phosphorylatable allele of Sic1 (Thr173A) display reduced viability in high osmolarity [39].

In addition to the direct phosphorylation of Sic1, Hog1 also regulates G1/S transition by the down-regulation of expression of G1 and S-phase cyclins (*CLN1*, *CLN2* and *CLB5* genes) [39,44]. The mechanism by which Hog1 represses the expression from MBF and SBF promoters remains to be elucidated. Mathematical modeling supported by quantitative in vivo experiments allowed to define and to quantify the temporal role and the direct contribution of the three individual components of the G1/S network controlled by the Hog1 SAPK [44]. These analyses showed that *CLB5* down-regulation is a key regulator in the G1 arrest. While down-regulation of *CLB5* is crucial during early and late G1, down-regulation of *CLN1,2* or stabilization of Sic1 seems to be important only important during late G1, when the Hog1 control over Clb5 is not sufficiently tight to prevent S-phase entry [44]. Therefore, the complex and strict Hog1 control over the G1/S network clearly

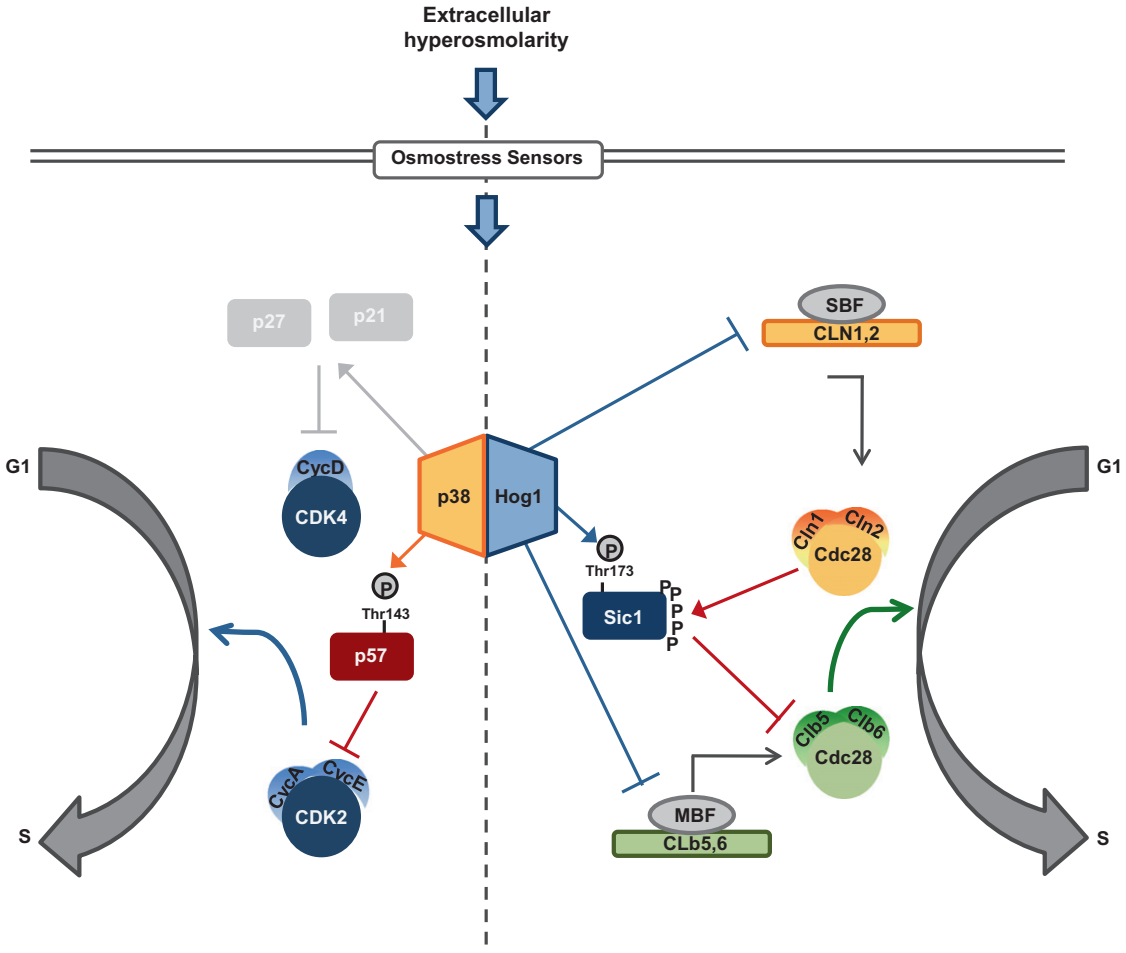

Fig. 2. G1-S control by p38/Hog1 SAPKs upon osmostress. Upon osmostress, activated p38 and Hog1 SAPKs phosphorylate the S/CDK inhibitor p57 or Sic1 respectively at one single residue. In mammalian cells (left panel), p57 phosphorylation on Thr143 leads to an increase of the affinity of p57 towards the Cyclin A/Cdk2 complex leading to a G1 arrest. In budding yeast (right panel) Sic1 phosphorylation on Thr173 inhibits its degradation by the proteasome which leads to a Sic1 with an increased stability resulting in G1 arrest. Hog1 activation also delays G1-S transition by down-regulating expression of *CLN*s and *CLB*s.

illustrates the necessity of cell adaptation to osmostress prior to re-enter cell cycle. The triple targeting of Clns, Clb5 and the CDK inhibitor Sic1 ensures a G1 transient arrest at any stage of G1.

In mammals, the protein network controlling the G1/S transition is orchestrated by the cyclin dependent kinases Cdk4,6-CyclinD and Cdk2/cyclin E, which regulate the expression, stability and activity of many cell cycle regulatory proteins. The activity of those CDK complexes is in turn regulated by two unrelated families of CDK inhibitors, the INK and the Cip/Kip CDKis [6,7]. The Cip/Kip family includes $p21^{CIP1}$, $p27^{KIP1}$ and $p57^{KIP2}$. Many reports have shown that the mammalian p38 SAPK is able to regulate several cell cycle phases in response to a variety of stresses [45,46]. For instance, DNA damage leads to a G1/S arrest through stabilization of CDK inhibitors $p21^{CIP1}$, $p27^{KIP1}$ [16,47,48]. Moreover, in response to environmental stresses such as osmostress or oxidative stress, p38 delays G1 progression by the direct targeting of the $p57^{KIP2}$ inhibitor [49]. Activated p38 phosphorylates a single residue (Thr143) of $p57^{KIP2}$ increasing its affinity towards Cdk2/CyclinA which leads to decrease on Cdk2 activity and the subsequent G1 arrest (see Fig. 2). Of note, cell cycle arrest mediated by the phosphorylation of p57 by p38 is essential for cell survival upon stress highlighting the relevance of this novel checkpoint pathway in mammals [49]. It is known that, upon osmostress, p38 orchestrates the transcription of hundreds of genes involved in cell proliferation and cell survival in a time dependent manner [50]. Thus, similarly that in yeast, G1 arrest might be important to provide time for cell adaptation to stress before re-entering into cell cycle.

4. Regulation of S-phase by SAPKs upon stress

The Hog1 SAPK is not only important to regulate the G1/S transition but it also plays a crucial role once the cells are already in S-phase to delay DNA replication in response to osmostress [51]. In budding yeast, S-phase is triggered by the coordinated function of the two essential S-phase kinase activities, S/CDK (Clb5,6-Cdc28) and DDK (Dbf4-Cdc7), which phosphorylate specific proteins in replication origins. DNA replication starts from multiple origins that are distributed all along the genome following a strict temporal program [52]. Thus, every origin has a specific time of activation which allows their classification into early origins (activated at early S-phase) or late origins (activated at late S-phase) [53]. The assembly of the protein complexes on the origins of replication is a highly regulated process that starts when cells exit mitosis. Initially, MCMs and the Cdc6 and Cdt1 proteins are bound to form the pre-replication complexes (pre-RC), which are not licensed for activation. During G1, other replication proteins such as Cdc45, Sld2, Sld3, Dpb11 and GINS are loaded to constitute the pre-IC (initiation complex). At this stage, the origins are already licensed to be activated by the S-phase kinase activities S/CDK and DDK. Those complexes phosphorylate specific target proteins on the pre-IC to induce full activation of the replicative helicase and polymerases to start DNA replication from every single origin. Cells have evolved a specific S-phase checkpoint to cope with multiple genotoxic agents that endanger the proper progression and completion of DNA replication. The S-phase checkpoint is mediated by Rad53 which safeguards DNA replication and preserves genomic integrity. Although it remains unclear the molecular mechanism by which Hog1 delays DNA replication, this function is clearly independent of the SAPK cell cycle targets Sic1 and Swe1 (see G1/S and G2/M sections respectively). In the presence of DNA damage or replication stresses, the Rad53-dependent checkpoint pathway delays S-phase by targeting Sld3 and Dbf4, which prevents the late origin firing [54–56]. It is worth noting that the cell has developed a sophisticate mechanism to inhibit the downstream functions of S/CDK by targeting its essential substrate Sld3, without altering the S/CDK activity, which would result in re-replication events and genomic instability [57]. Strikingly, Hog1-dependent arrest in S-phase upon osmostress is independent of the known Rad53-dependent checkpoint pathway, suggesting that there must be a novel S-phase checkpoint pathway that delays DNA replication in the absence of DNA damage or replication stress [51]. It will be interesting to explore whether Hog1 utilizes the same smart mechanism to inhibit progression through S-phase or the SAPK signaling pathway has elaborated a novel mechanism to block DNA replication.

Why cells need to block S-phase progression upon osmostress is a key question that remains to be elucidated. In response to osmostress, Hog1 orchestrates a fast and transient activation of transcription of hundreds of stress-responsive genes essential for adaptation to stress (reviewed in [15]). Induction of gene expression might represent an important drawback during in S-phase, since the risk of collision between the replication and the transcription machineries significantly increases. Actually, it has been shown that collision between RNA Pol II and DNA polymerase induces transcription-associated recombination [58]. Therefore, Hog1-dependent S-phase arrest might be essential to permit the proper adaptive response and to protect genomic stability.

S-phase entry and progression in mammals is also controlled by the corresponding S/CDK and DDK activities [59]. Although the precise targets that permit origin activation are not elucidated yet, the main processes governing DNA replication is highly conserved from yeast to mammals [59,60]. Recently, it has been reported that there are early and late replication origins in the genome of higher eukaryotic cells, indicating that DNA replication in mammals also follows a strict temporal pattern of origin activation. Moreover, upon genotoxic stress, mammalian cells block late origin firing, confirming the high homology in all mechanisms controlling DNA replication [61]. Up to now is not known whether exist an osmostress-dependent S-phase checkpoint in mammals. However, it has been recently reported a novel role for the p38 SAPK in regulating origin licensing during G2–M [62]. Prior to S-phase, during M and G1, a strict regulation of origin licensing ensures that every active origin of replication fires once and only once in each cell cycle. Recruitment of the pre-RC protein Cdt1 at origins during M-phase is essential for MCM loading during G1, which is necessary and sufficient for origin licensing [63,64]. Actually, failure to control MCM loading properly might cause insufficient origin licensing during G1 or inappropriate origin relicensing after the onset of S-phase, leading to replication errors and genomic instability [65]. Phosphorylation of Cdt1 by p38 decreases loading of MCM, inhibits origin firing, as well as it results in an increased stability of Cdt1. Although being apparently contradictory, this mechanism would allow the cell to have a sufficient pool of inactive Cdt1 to rapidly modulate the origin licensing and activation according to the necessities of the cell. Therefore, as in budding yeast, mammalian cells would also adequate DNA replication to cell adaptation upon osmostress, presumably to preserve genomic integrity.

5. Regulation of G2 by SAPKs upon stress

Upon osmostress, Hog1 not only cotros G1/S but also G2/M. Here, Hog1 activation stabilizes the cell cycle inhibitor Swe1 and down-regulates G2 cyclin (*CLB2*) transcription [20,66,67] (see Fig. 3). Entry into mitosis is driven by the activity of Clb2–Cdc28, which is tightly regulated by Swe1. Swe1 degradation basically depends on two independent mechanisms; the phosphorylation by Clb2–Cdc28 [68,69] and its degradation by the Hsl1 and Cdc5 kinases [68,70]. When bound to septins, Hsl1 tethers the adaptor protein Hsl7 to the bud neck, which is in turn required for Swe1 recruitment. Once in G2, Swe1 is found at the septin ring, which

is then targeted by Cdc5 that leads to Swe1 degradation and relieve of Clb2–Cdc28 inhibition [71,72]. Thus, the tight regulation of Swe1 phosphorylation and subsequent degradation is critical for the timely activation of the Clb2–Cdc28 complex and ensures the proper assembly of the septin ring throughout G2/M transition. Swe1 has been involved in other cell cycle checkpoints such as the morphogenesis checkpoint, where Swe1 targets Cdc28–Clb2 activity to delay entry into mitosis when the critical cell size has not been reached [38,73,74].

While the mechanism by which Hog1 down-regulate *CLB2* transcription is not known, it is well established how Hog1 regulates Swe1 [20,66]. Upon osmostress, Hog1 directly phosphorylates the Hsl1 kinase, which delocalizes Hsl7 and impairs Swe1 recruitment to the bud neck, preventing its degradation. The resulting Swe1 accumulation and the consequent reduction in Clb2–Cdc28 activity leads to a G2 transient arrest. Of note, mutants that cannot accumulate Swe1 fail to arrest in G2 and render cells osmosensitive. Moreover, the combined deletion of *SIC1* and *SWE1* results in a synergistic osmosensitivity phenotype [66], highlighting the relevance of cell cycle control upon osmostress at different stages of the cell cycle.

In mammals, ionizing radiation and UV light stimuli trigger the activation of the G2–M checkpoint. Those cellular insults generate specific aberrant structures in the DNA that lead to activation of the ATM/ATR checkpoint kinases [75]. Once activated, they transduce the signal to the final effector kinases Chk1 and Chk2 which lead to the cell cycle arrest. The main mechanism by which these kinases mediate G2–M arrest is through the inhibitory phosphorylation of the Cdc25 family of phosphatases, which are well known positive regulators of the Cyclin/Cdk complexes [76]. Interestingly, the p38 SAPK has been also involved in the G2–M checkpoint in response to DNA damage. In response to DNA damage (e.g. UV, genotoxic stress.), cells activate a complex kinase-based signaling network to arrest the cell cycle and initiate DNA repair. p38 regulates p53, but also in p53-defective tumor cells rewire their checkpoint response and become dependent on the p38/MK2 pathway for survival after DNA damage, despite a functional ATR-Chk1 pathway [77–79].

Despite the relevance of p38 in response to DNA damage, little is known about its role upon osmostress. It has been reported that upon hyperosmotic shock, cells undergo a G2 arrest preventing M-phase entry. Therefore, suggesting that p38 might be somehow involved in the initiation of this G2–M checkpoint. In the absence of p38 activity, cells significantly abrogate the G2 arrest upon osmostress and display high levels of DNA damage [22,80]. Thus, as in yeast, p38 SAPK also controls G2/M transition.

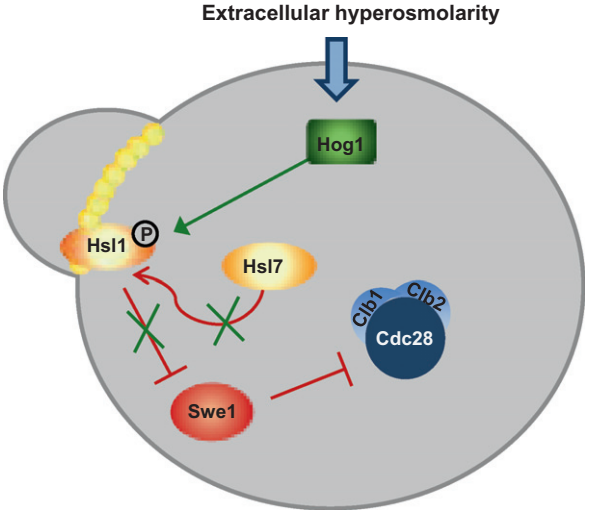

Fig. 3. Hog1 mediated arrest at G2. In G2, Swe1 is recruited to the septin ring at the bud neck (yellow circles) by Hsl1–Hsl7. Recruitment of Swe1 allows its phosphorylation by Cdc5 and its subsequent degradation, relieving Clb2–Cdc28 inhibition. Upon osmostress, Hog1 phosphorylates Hsl1 which delocalize Hsl7, and thus, Swe1 cannot be tethered at the bud neck and cannot be degraded. This results in a persistent inhibition of Clb2–Cdc28 which leads to a G2–M arrest.

6. Conclusions

Activation of SAPKs results in the generation of a set of adaptive responses that leads to the modulation of several aspects of cell physiology essential for cell survival upon stress. Together with the control of cell metabolism and the reorganization of gene expression, a major adaptive response consists in the modulation of cell cycle progression. The coordination of all those events within a very limited time-frame is essential to preserve genomic integrity and guarantee cell viability upon a sudden change on the environmental conditions and it can only be achieved by the integrated control of those aspects. SAPKs play a key role in transducing stress signals and to mount the appropriate responses to stress. Genetic inactivation of Hog1 in yeast or p38 in mammals results in cells that are not competent to adapt when exposed to stress.

Cells arrest cell cycle progression rapidly in response to stress. Deficiencies in delaying cell cycle upon stress, as found in *hog1* or $p38^{-/-}$ cells, lead to compromised cell viability. Thus, the role of cell cycle control is critical in maximizing cell viability upon stress. The Hog1 SAPK controls G1 and G2 transitions by complementary mechanisms that involve the stabilization of cell cycle inhibitors and the down-regulation of cyclin expression. Similarly, in mammals, p38 SAPK also controls CDK inhibitors and the levels of cyclin expression. Therefore, the number and variety of the mechanisms controlled by those SAPKs indicates the relevance of a thigh control of cell cycle progression upon stress. It will be interesting to further explore whether Hog1 and p38 control other cell cycle related processes through specific and sophisticated mechanisms, similar to that of p38 regulation of origin licensing through Cdt1.

Proper adaptation seems to be a pre-requisite for advancing to the following cell cycle stage and thus, it seems reasonable that the same signal transducing kinase is able to exert control in different cell cycle phases. Both Hog1 and p38 act on several cell cycle phases in response to stress. This regulation might be critical to guarantee that cells at any stage of the cell cycle are competent to delay cell cycle and mount the appropriate adaptive responses.

Acknowledgements

We apologize to colleagues in the field for not citing all relevant papers owing to space constraints. The laboratory of FP and EN is supported by grants from the Spanish Government (BIO2009-07762 to FP, BFU2011-26722 to EN), Consolider Ingenio 2010 programme CSD2007-0015, FP7 UNICELLSYS grant (#201142) and the Fundación Marcelino Botín (FMB) to FP. FP and EN are recipients of an ICREA Acadèmia (Generalitat de Catalunya).

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